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(54) Title: ANTITHROMBOTIC ORGANIC NITRATES

(57) Abstract

Compounds, or their compositions, of the general formula: $A-(X_1-NO_2)_{to}$ or their salts, for the preparation of medicaments for antithrombotic application, where: to is an integer equal to 1 or 2; X_1 is an alkylene connecting bridge, A is the residue of cardiovascular products, preferably timolol or enalapril.

ANTITHROMBOTIC ORGANIC NITRATES

The present invention relates to new products having an antithrombotic activity.

Cyclooxygenase (COX)-inhibiting anti-inflammatory products are known from previous patent applications in the name of the Applicant. See in particular the published patent applications WO 94/04484, WO 94/12463, WO 95/09831, WO95/30641. These patent applications referred to non-steroid anti-inflammatory products with a non-acid ending and to those with an acid ending mentioned as products known in the art.

Said products showed a much lower toxicity level compared to the reference products not containing group $-ONO_2$.

The need for available products having an antithrombotic activity combined with lower toxicity in long term treatment was felt. In particular, the efficacy and safety of antithrombotic agents are closely related and research is aiming to find out new molecules with an increased therapeutic index, i.e. with improved efficacy and reduced toxicity (Goodman & Gilman: "The pharmacological basis of therapeutics", Ed. J. Hardman, L. Limbrid, page 1357, 1996).

It was unexpectedly and surprisingly found that the products of the invention as defined below are effective in inhibiting platelet aggregation induced by different kinds

of stimuli, in particular collagen and thrombin, and at the same time exhibit high safety in general, in particular a high gastric safety, without causing lesions to the gastro-intestinal mucosa in the treated animals.

The results of the present invention are much more sur-prising considering that the new classes of products of the invention are not cyclooxigenase (COX) inhibiting products and, therefore, they cannot be drawn in any way from the products described in the known art, in particular in the above patents.

A subject of the present invention are the compounds, or their compositions, of the general formula:

$$A - (X_1-NO_2)_{t_o}$$
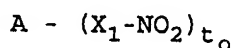
or their salts, for use as medicaments, in particular as antithrombotic agents since they are effective in inhibiting platelet aggregation, where:

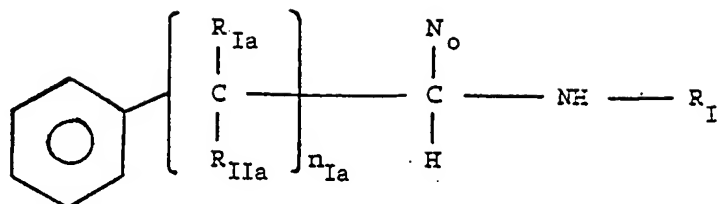t_o is an integer equal to 1 or 2;

$A = RN_o$ where $N_o = (COX_u)_t$- or $COON_1$ where t is an integer equal to zero or 1; u is an integer equal to 0 or 1;

$X = O$, NH, NR_{1c} where R_{1c} is a linear or branched alkyl ha-ving from 1 to 10 carbon atoms; N_1 is a linear or branched alkyl having from 1 to 10 carbon atoms or hydrogen;

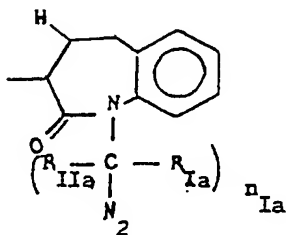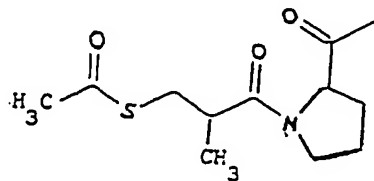R is chosen from the following groups:

2

* Group A)

Ia)

where R_{Ia} and R_{IIa} are equal or different one from the other
and are H or a linear or whenever possible branched alkyl
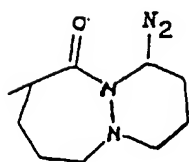having from 1 to 3 C atoms, preferably $R_{Ia} = R_{IIa} = H$; n_{Ia} is
an integer from 1 to 6, preferably from 2 to 4;

R_I can be:

(X)

(XI)

(XII)

(XIII)

3
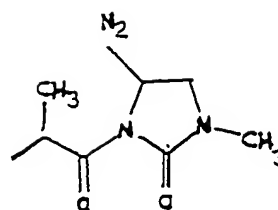
(XIV)

(XV)

(XVI)

(XVII)

(XVIII)

(XIX)

$(R_{IIa}-C-R_{Ia})_{n_{Ia}}$

(XX)

(XXI)

where N_2 has the same meaning as N_O; at least one of the

groups N_O or N_2 having one free valence capable of binding to

X_1, (that is, $t = 1$),

Ib)

R_{Ia}, R_{IIa}, n_{Ia} are as defined in Ia;

N_3 is H, $(CH_3)_2CH-CH-OCOCH_2CH_3$, or a free valence to which X_1

binds (that is, N_3 is absent);

R_{Ib} is chosen from:

VI)

V)

N_2 is as above defined, where at least one of the groups N_3

or N_2 has a free valence capable of binding to X_1 (when it is

N_2, $t = 1$);

5

N_2, t = 1);

Ic) where t = 1

where N_o is as above defined where t = 1, i.e. it has a free

valence capable of binding to X_1;

R_{Ic} is chosen from H, $-COCH_3$, or

(VII)

Id)

where N_2 is as defined, and at least one of the groups N_2 has

a free valence (t = 1) capable of binding to X_1;

* Group B

where t = 1 and u = 0;

IIa)

where R_{Ia}, R_{IIa} are as defined in Ia);

R_{IIb} has the meaning of R_{Ia};

R_{BA} is chosen from:

L)

LI)

LII)

LIII)

LIV)

LV)

7

LVI)

LVII)

LVIII)

LIX)

IIb)

where, in group B), N_2 is as above defined and at least one

of the N_2 groups has a free valence capable of binding to X_1,

(that is, at least one N_2 substituent has t = 1;

X_1 is a bivalent connecting bridge chosen from the following:

-     YO   where Y is a linear or whenever possible branched

      C_1-C_{20} alkylene, preferably having from 2 to 5 carbon

      atoms, or an optionally substituted cycloalkylene ha-

      ving from 5 to 7 carbon atoms;

-     Y_1 chosen from

      -

8

where n_3 is an integer from 0 to 3;

$$CH_2O-$$

COOH CH_2-

- $-(CH_2-CH-CH_2-O)_{nf'}-$

  ONO_2

where nf' is an integer from 1 to 6, preferably from 2 to 4;

- $-(CH-CH_2-O)_{nf}-$

  R_{1f}

where R_{1f} = H, CH_3 and nf is an integer from 1 to 6; preferably from 2 to 4.

The compounds which may be mentioned, and which are the preferred compounds, are those listed below where R can be obtained by the processes known in the art.

For example, the compounds and processes described in The Merck Index, Ed. 12 of 1996, herein fully incorporated by reference, can be mentioned as precursors and related processes. The precursors (according to the Merck nomenclature) as those shown below, where the various substituents shown in the formulas of group A) and group B) are as defined in the compounds listed: Alacepril, Benazepril, Captopril, Ceronapril, Cilazapril, Delapril, Enalapril, Enalapri-

lat, Fosinapril, Imidapril, Lisinopril, Quinapril, Ramipril, Spirapril, Temocapril, Trandolapril, Moveltilpril, Perindopril, Befunolol, Betaxolol, Bupranolol, Carteolol, Levobunolol, Metipranolol, Timolol, Oxprenolol, Mepindolol, Atenolol, Labetalol.

The connecting bridges X_1 as above defined can be obtained using the methods from the known art or modifying the known methods by introducing X_1 bridges when these are different from the connecting bridges described in the mentioned patents by processes known in the art. In general, the connection between A and X_1 is, as seen, of an ester or amide type (NH or NR_{1c}, as defined in X). Any synthetic route well known for forming these bonds can be used.

In the case of esters, the most direct synthetic route includes reaction of acyl chlorides A-CO-Cl, or A-(CO-Cl)$_2$, in halogen alcohols of the type HO-Y_a-Cl, HO-Y_a-Br, HO-Y_a-I, where Y_a is equal to Y or Y_1 as above defined without the oxygen atom —O-, in experimental conditions which are part of the known art.

The reaction products of formula A-CO-O-Y_a-Cl(Br,I) can also be obtained by reacting the sodium or potassium salts of said acids A-CO-OH with di-halogen derivatives of the general formula Y_aCl_2, Y_aBr_2 or Y_aI_2.

The reaction products are converted into the final pro-

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ducts by reaction with $AgNO_3$ in acetonitrile according to processes known in the prior art.

The general scheme is as follows:

$A-CO-Cl + HO-Y_a-Br$ ------> $A-CO-O-Y_a-Br + AgNO_3$ ----->

$A-X_1NO_2$
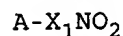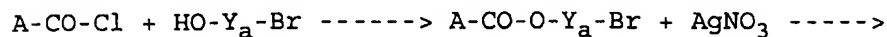
where $X_1 = Y_aO$.

The general scheme can also be as follows:

$A-CO-ONa+Br_2Y_a$ -----> $A-CO-O-Y_a-Br + AgNO_3$ ------> $A-X_1NO_2$
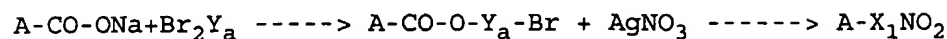
where $X_1 = Y_aO$.

In the case of amides, the synthetic sequence includes reaction of the same acyl chlorides $A-CO-Cl$ with amino alcohols of the general formula NH_2-Y_a-OH or $NHR_{1c}-Y_a-OH$ to give amides of the general formula:

$A-CO-NH-Y_a-OH$ or $A-CO-NR_{1c}-Y_a-OH$
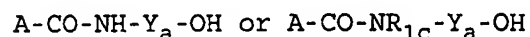
in accordance with known methods.

Reaction of these amides with halogenating agents such as, for example, PCl_5, PBr_3, $SOCl_2$, etc, leads to halogen derivatives of the general formula:

$A-CO-NH-Y_a-Br(Cl)$ and $A-CO-NR_{1c}-Y_a-Br(Cl)$.
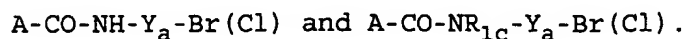
By reaction with $AgNO_3$ in acetonitrile according to known literature methods said latter products lead to the final products AX_1NO_2.

The sequence may be represented as follows:

$A-CO-Cl + NHR_{1c}-Y_a-OH$ -----> $A-CO-NR_{1c}-Y_a-OH$ $\xrightarrow{PCl_5}$

$A-CO-NR_{1c}-Y_a-Br + AgNO_3$ -----> $A-CO-NR1_{1c}-Y_a-ONO_2$
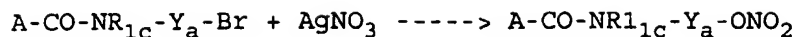
where Y_aO is X_1.

An alternative route to ester formation is reaction of the sodium or potassium salts of acids with the nitric esters of halogen alcohols of the general formula:

$$NO_2-O-Y_a-Cl(Br,I)$$
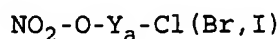
to give directly the products of the invention.

The reaction scheme is as follows:

$A-CO-ONa + Br-Y_a-ONO_2$ ------> $A-CO-O-Y_a-ONO_2$
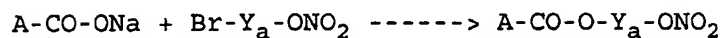
where Y_aO is X_1.

Other synthetic routes similar to those described above are the ones where dihalogen derivative Br_2Y_a is reacted with enolates. The reaction products are then converted by reaction with $AgNO_3$ in acetonitrile according to the above reaction. The general scheme shown for an -OH belonging to group A is as follows:

$$-ONa + Br_2-Y_a \xrightarrow{\hspace{2cm}} -O-Y_a-Br \xrightarrow{AgNO_3} -O-Y_a-ONO_2$$
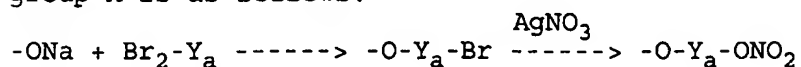
A general method for the -OH group is described in Example 1 only for illustrative purposes.

The processes to obtain these connecting groups X_1 are described in patent application WO 95/30641 herein fully incorporated by reference.

The products of the invention as described above are novel as medicaments in general. In particular they are no-

vel for their antithrombotic activity and are also novel as compounds as such.

Additional pharmaceutical uses which can be mentioned for the products of the invention are, for example, their antihypertensive activity (e.g. arterial hypertension, glaucoma) and their cardioprotective activity (e.g. angina pectoris, cardiac failure, coronary ischaemia).

As to antihypertensive activity, it should be noted that the products of the invention showed an extremely satisfactory pharmaco-therapeutic profile with improved efficacy compared to the precursors which do not contain group $-ONO_2$ and, at the same time, showed superior safety.

It should also be noted that the products of the invention exhibit an antihypertensive activity combined with an antithrombotic activity. This is an outstanding benefit in the treatment of cardiovascular disease in general since the purpose of any therapeutical approach is to ensure to the patient an altogether reduced risk of cardiovascular disease, such as myocardial or cerebral infarction and atherosclerosis (Goodman & Gilman "The pharmacological basis of therapeutics", Ed.J.Hardman, L. Limbrid, pages 747, 1354-7, 1996).

The following examples are being provided as an explanation not a limitation of the present invention.

EXAMPLES

EXAMPLE 1: Chemical synthesis and characterization of NO-ti-molol (NO-TIM)

Synthesis of (R)-(4-nitroxy)butanoate of 1-[(1,1-dime-thyl)amino]-3-{[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy}-2-propyl maleate.

The starting point is timolol maleate (a commercial product), the timolol having the general formula

a)
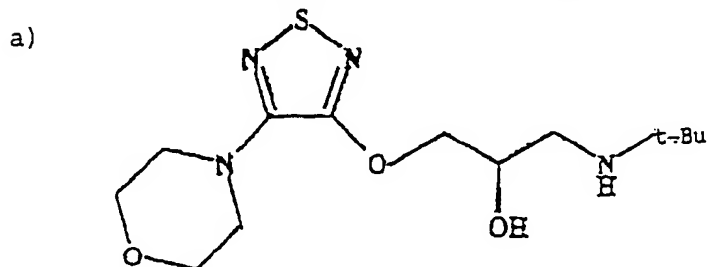
(S)-1-[(1,1-dimethylethyl)amino]-3-{[4-morpholinyl]-1,2,5-thiadiazol-3yl]oxy}-2-propanol.

Timolol maleate (2.0 g) was treated with a solution of 10% NaOH (30 ml). 30 ml of CH_2Cl_2 were added and then the phases were separated. The aqueous phase was extracted several times with CH_2Cl_2. The pooled organic phases were dried (Na_2SO_4) and the solvent evaporated at reduced pressure. 1.4 g of pure product were obtained (yield 96%).

^{1}H NMR (300 MHz CDCl$_3$:δ 1.05 (9H,s,3CH$_3$), 2.7 (2H, 2dd, CH$_2$-NH), 3.5 (4H, m, morpholine), 3,8 (4H, t, morpholine), 3.85 (1H, m, CH), 4.4 (2H, 2dd, O-CH$_2$).

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b)
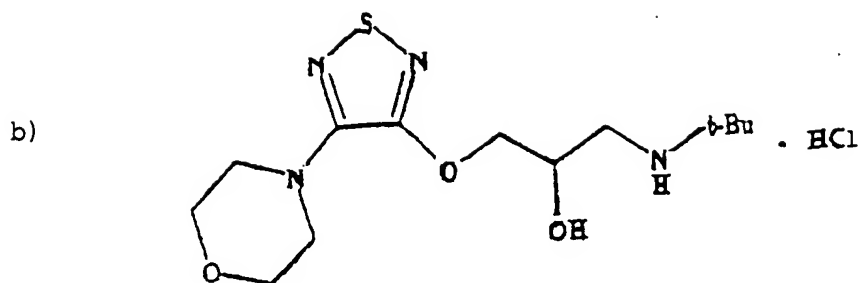
(S)-1-[(1,1-dimethylethyl)amino]-3-{[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy}-2-propanol hydrochloride.

0.8 ml of a 7M HCl solution in isopropanol was added dropwise to a magnetically stirred solution of timolol (1.4 g) in isopropanol (30 ml). The solution was stirred for 30 minutes. The reaction mixture was freed of the solvent at reduced pressure. 1.47 g of pure product was obtained (yield 91%).

^{1}H NMR (300 MHz CDCl$_3$): δ 1.45 (9H,s,3CH$_3$), 3.05 (2H, 2dd, CH$_2$-NH), 3.5(4H,t,morpholine), 3.8 (4H,t,morpholine), 4.5 (2H,d,O-CH$_2$), 4.55 (1H,m,CH).

c)
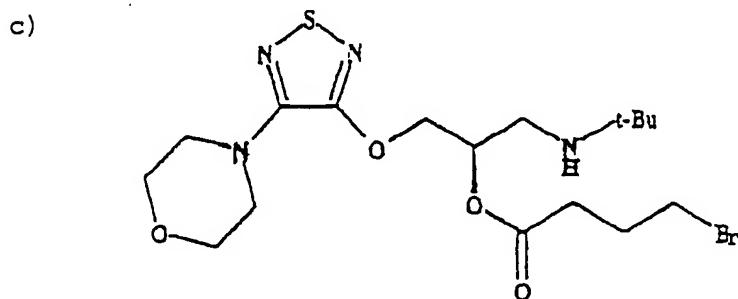
(R)-(4-bromo)butanoate of 1-[(1,1-dimethylethyl)amino]-3-{[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxyl}-2-propyl

4-Bromobutyryl chloride (0.4 ml) was added dropwise in a nitrogen atmosphere to a magnetically stirred solution of timolol hydrochloride (0.82 g) in CHCl$_3$ dried over P$_2$O$_5$

(20 ml). Stirring was continued for 4 days. The reaction mixture was then freed of the solvent at reduced pressure. The residue was chromatographed on silica gel using diethyl ether with 3% Et_3N as an eluant. 0.830 g of pure product was obtained from the intermediate fractions (yield 78%).

^{1}H NMR (300 MHz $CDCl_3$):δ 1.05 (9H,s,3-CH_3), 2.05 (2H,m,$COCH_2$-CH_2-CH_2-ONO_2), 2.5 (2H,m,$COCH_2$-CH_2CH_2-ONO_2), 2.8(2H,d,CH_2-NH), 3.5 (6H,m,morpholine, CH_2-Br), 3.8 (4H,t,morpholine), 4.65 (2H, 2dd, O-CH_2), 5.25 (1H,m,CH).

d)
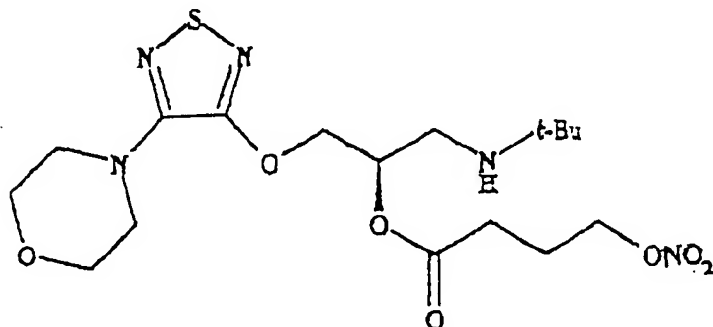
(R)-(nitroxy)butanoate of 1-[(1,1-dimethylethyl)amino]-3-{[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy}-2-propyl.

A solution of $AgNO_3$ (0.450 g) in CH_3CN (5 ml)was added dropwise at ambient temperature to a magnetically stirred solution of timolol (4-bromo)butanoate (0.830 g) in CH_3CN (10 ml). The temperature was progressively raised up to 60°C and reaction was continued for 24 hours. The reaction mixture was freed of the solvent at reduced pressure. The residue

was chromatographed on silica gel using diethyl ether with 3% Et_3N as an eluant. 0.51 g of pure product was obtained from the first fractions (yield 64%)

^{1}H NMR (300 MHz $CDCL_3$): δ 1.05 (9H,s,3CH$_3$), 2.05 (2H, m,$COCH_2$-CH_2-CH_2-ONO_2), 2.5 (2H, 2t, $COCH_2$-CH_2-CH_2-ONO_2), 2.8 (2H,d,CH_2-NH), 3.5 (4H,m,morpholine), 3.8 (4H,t,morpholine), 4.5 (2H,t,-CH_2-ONO_2), 4.58 (2H, 2dd, O-CH_2), 5.25 (1H, m,CH). MS:M$^+$ 448

e)
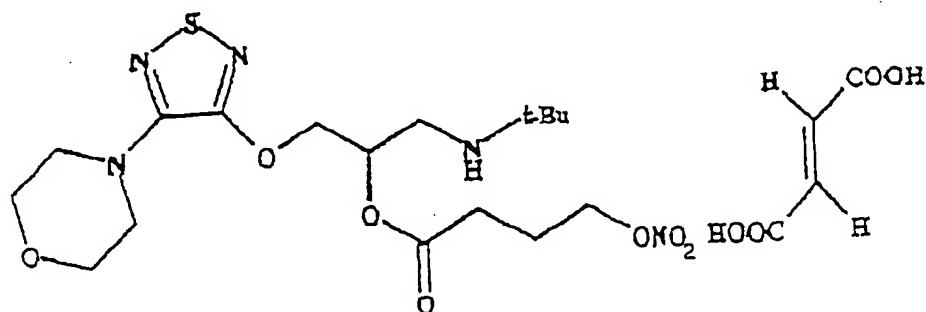
(R)-(4-nitroxy)butanoate of 1-[(1,1-dimethylethyl)amino]-3-{[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy}-2-propyl maleate.

A solution of maleic acid (0.132 g) in acetone (5 ml) was added dropwise to a magnetically stirred solution of timolol (4-nitroxy)butanoate (0.50 g) in acetone (10 ml). Stirring was continued for 2 hours. The reaction mixture was freed of the solvent at reduced pressure. The crude residue was grounded with diethyl ether to give 0.5 g of a white solid (m.p. 133-136°C, yield 70%)

^{1}H NMR (300 MHz $CDCl_3$): δ 1.48 (9H,s,3CH$_3$), 2.05 (2H,m,-

COCH$_2$-CH$_2$-CH$_2$-ONO$_2$),   2.58   (2H,   2td,   COCH$_2$-CH$_2$-CH$_2$-ONO$_2$),

3.3 (2H,2m, CH$_2$-NH$_2$), 3.5 (4H, m, morpholine), 3.8 (4H ,t,

morpholine), 4.5 (2H, t, CH$_2$-ONO$_2$)   4.7(2H, 2dd, O-CH$_2$),

5.55 (1H, m, CH), 6,47 (2H, s, maleic)

EXAMPLE 2A: Chemical synthesis and characterization of NO-enalapril (NO-ENA)

The reaction scheme is as follows:
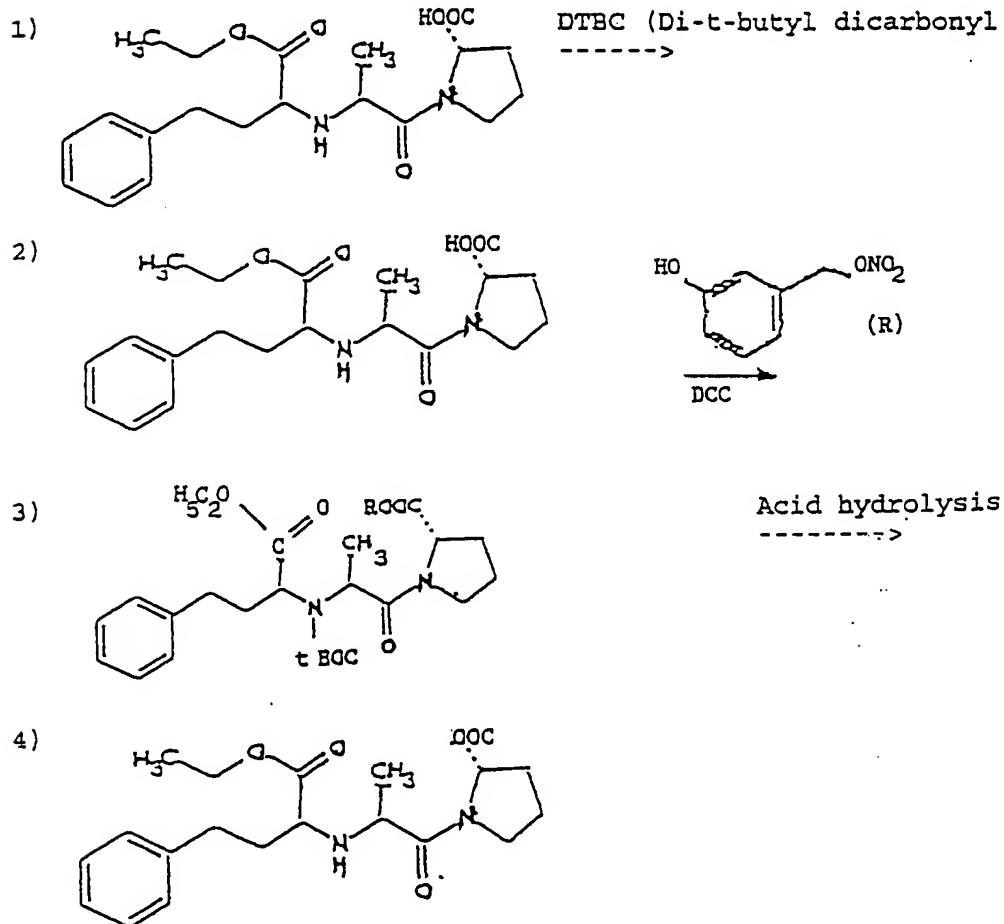
1)   DTBC (Di-t-butyl dicarbonyl
                                                             ------->

2)

3)   Acid hydrolysis
                                                             -------->

4)

Step 1

3 g of diterbutyldicarbonyl (DTBC) was added at ambient tem-

perature to a solution of 5 g of enalapril in 100 ml of di-methylformamide (DMF) and triethylamine (TEA) (2.76 g). The solution was stirred for 16 hours. Then the solution was washed twice with diluted HCl and water, extracted 3 times with 100 ml portions of ether. The dried and evaporated-off organic phases gave 3 g of a formula 2) product (an oil). In formula 2) tBOC = t-butyldicarbonyl.

Step 2

1.4 g of dicyclohexyl carbodiimide (DCC), and then 30 ml of a solution of 1.1 g of nitroxymethylphenol in CH_2Cl_2, were added to 3 g of N-protected enalapril (a compound of formula 2) dissolved in 50 ml of methylene dichloride. The mixture was stirred overnight, dicyclohexylurea was filtered off and the solvent was evaporated off the dryness. The residue was chromatographed on silica gel 60 Merck using an ethyl acetate/hexane mixture. A fraction of 2 g of intermediate of formula 3), where R was the residue of nitroxymethylphenol without OH, was collected.

Step 3

1 g of the product of formula 3) was dissolved at 0°C in a 4N solution of 30 ml of dry HCl gas in ethyl acetate (ACO-Et) and stirred for 10 hours. The precipitate obtained was filtered and dried under vacuum 0.5 g of a product 4) was obtained.

EXAMPLE 28: Chemical synthesis and characterisation of NO-enalaprilate (NO-ENP)

The reaction scheme is as follows:

1)

DTBC (Di-t-butyl dicarbonyl)
$\longrightarrow$

2)

(R)

DCC
$\longrightarrow$

3)

Acid hydrolysis
- - - - - - - >

4)

Step 1:

3 g of diterbutyldicarbonyl (DTBC) was added at ambient temperature to a solution of 5 g of enalaprilate in 100 ml of dimethylformamide (DMF) and triethylamine (TEA) (2.76 g). The solution was stirred for 16 hours. Then the solution

was washed twice with diluted HCl and water, extracted 3
times with 100 ml portions of ether. The dried and evapora-
ted-off organic phases gave 3 g of a product 2) as an oil.
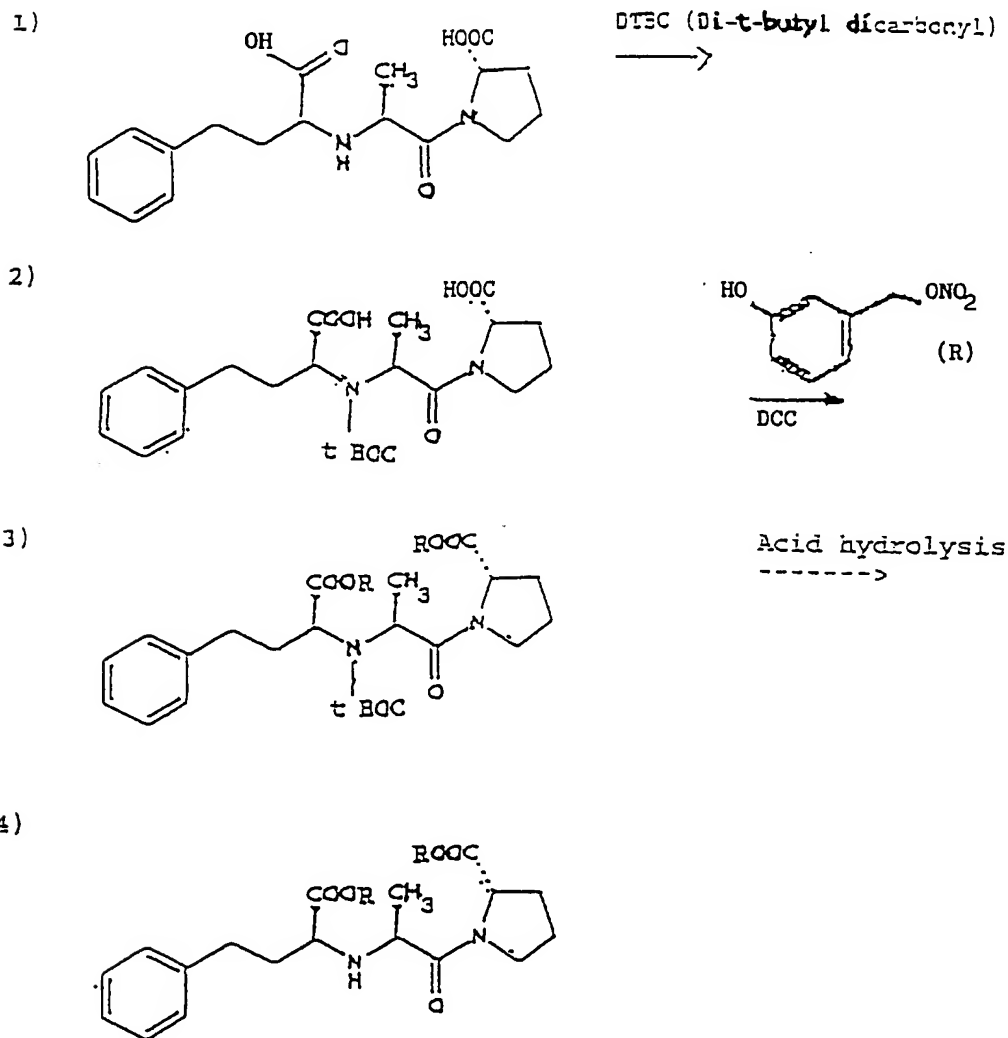
In formula 2) tBOC = t-butyldicarbonyl.

Step 2:

2.75 g of dicyclohexyl carbodiimide (DCC), and then 30 ml of
a solution of 2.25 g of nitroxymethylphenol, were added to
3 g of N-protected enalaprilate dissolved in 50 ml of methy-
lene dichloride. The mixture was stirred overnight, dicyclo-
hexylurea was filtered off and the solvent was evaporated
off to dryness. The residue was chromatographed on silica
gel 60 Merck using an ethylacetate/hexane mixture. A
fraction of 3 g of intermediate product 3) was collected.

R has the meaning as defined in Example 2A.

Step 3:

1 g of product 3) was dissolved at 0°C in a 4N solution of
30 ml of dry HCl gas in ACOEt and stirred for 10 hours. The
precipitate obtained was filtered and dried under vacuum.

0.7 g of a product 4) was obtained.

EXAMPLE 3: Pharmacological studies

The products from Examples 1 and 2 had been administe-
red in vivo always as 2%-by-weight suspensions in carboxy-
methyl cellulose.

The experimental groups were made up of 6 to 8 samples

to allow appropriate statistical evaluation, which was car-
ried out when needed.

As far as acute toxicity for the compounds which are
the object of the invention, it was evaluated after a single
oral dose to groups of 10 mice each.

Death rate and presence of toxic symptoms were recorded
during an observation period of 14 days. Even after a 50
mg/kg dose the animals showed no sign of apparent overt to-
xicity.

EXAMPLE 3A

STUDY OF ANTIPLATELET ACTIVITY

The ability of NO-ENA and NO-TIM to inhibit platelet
aggregation was evaluated using an in vivo model as descri-
bed by Pinon (J. Pharmacol. Methods 12,79,1989). 5 groups of
male Wistar rats (200 to 250 g) received an oral daily dose
of 10 mg/kg of respectively, NO-ENA, enalapril, NO-TIM, ti-
molol or vehicle for 5 days. At an appropriate time on the
forth day food (but not water) was withdrawn. 18 to 20 hours
later the animals received the last treatment. One hour la-
ter the animals were anaesthetized with 10% urethane (1
mg/kg intraperitoneally) and the left jugular vein and right
carotid artery were incannulated. Collagen (type 6, Sigma)
was then administered intravenously at a dose of 2 mg/kg.
Three minutes later two blood samples (A and B) were colle-

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cted from the carotid artery using 2.5-ml plastic syringes
in the following manner: sample A, 0.4 ml of blood in 1.6 ml
of EDTA/formalin buffer (ETDA tetrasodium salt 24 mM, KH_2PO_4
1.3 mM, Na_2PO_4 13.4 mM), the samples were then transferred
into 5-ml polystyrene test tubes and allowed to settle for
15 minutes at ambient temperature. After this time, the pla-
telet aggregations in sample A were fixed in formalin, while
those from sample B were treated with EDTA. Platelet count
was then made in each sample using a conventional microsco-
pe. The count for sample B was the total number of plate-
lets, while for sample A were considered only non-aggregated
platelets. The results were expressed as per-cent aggrega-
tion, calculated as follows: {[1-(platelet count in sample
A)/(platelet count in sample B)] x 100}. The results were
expressed as per-cent inhibition of the control group (vehi-
cle) and shown in Table 1.

TABLE 1

STUDY OF ANTIPLATELET ACTIVITY OF NO-ENA OR NO-TIM VERSUS

ENALAPRIL OR TIMOLOL IN RATS

COMPOUND	ANTIPLATELET ACTIVITY (%)
NO-ENA	65
NO-TIM	58
ENALAPRIL	15
TIMOLOL	2

As shown in Table 1, differently from the reference pro-
ducts, the nitroderivatives of the invention were able to
inhibit aggregation induced by collagen.

EXAMPLE 3B: STUDY OF ANTITHROMBOTIC ACTIVITY

5 groups of male Charles River rats of the Swiss str-
ain, 15 to 20 g, received a daily oral dose of 10 mg/kg of,
respectively, NO-ENA, enalapril, NO-TIM, timolol or vehicle
for 5 days. At an appropriate time on the fourth day food
(but not water) was withdrawn. 18 to 20 hours later the ani-
mals received the last treatment. One hour later the animals
were injected into the caudal vein with 0.1 ml of a collagen
(type 6, Sigma) mixture plus adrenaline hydrochloride (100
μM) diluted in a solution of 0.154 M sodium chloride. As
previously explained (Cirino G. et al., Thrombosis Reasearch
79, 73, 1995), injection of this mixture caused death within
3 minutes in 90% of the control animals.

The results were expressed as inhibition percentage compared

to the control group and are shown in Table 2.

TABLE 2

STUDY OF ANTITHROMBOTIC ACTIVITY OF NO-ENA OR NO-TIM VERSUS

ENALAPRIL OR TIMOLOL IN RATS

COMPOUND	ANTITHROMBOTIC ACTIVITY (%)
NO-ENA	53
NO-TIM	44
ENALAPRIL	11
TIMOLOL	6

As shown in Table 2, differently from the reference

products, the nitroderivatives of the invention were able to

inhibit thrombosis induced by collagen.

EXAMPLE 3C: STUDY OF ANTIHYPERTENSIVE ACTIVITY

The ability of NO-ENA to inhibit hypertension was eva-

luated using an in vivo model as described by Ribeiro et al.

(Hypertension 20, 298, 1992). 5 groups of male Wistar rats

(235 to 284 g) received a daily intravenous dose of 10 mg/kg

of, respectively, NO-ENA, enalapril, NO-TIM, timolol or ve-

hicle for 5 days. Arterial hypertension was induced by admi-

nistration of N^w-nitro-L-argininemethyl ester (L-NAME) in the

drinking water for 6 weeks. L-NAME was dissolved in the

drinking water at a concentration of 60 to 70 mg 100 ml^{-1} so

as to administer a daily amount of about 60 mg kg^{-1}. One

hour after treatment the systemic blood pressure was measu-

red by the tail-cap method (Zats, Lab. Anim. Sci.42, 198,

1990).

TABLE 3

STUDY OF ANTIHYPERTENSIVE ACTIVITY OF NO-ENA VERSUS ENALA-

PRIL IN RATS

COMPOUND	MEAN BLOOD PRESSURE (mmHg)
VEHICLE	170 ± 7
NO-ENA	115 ± 4 [*]
ENALAPRIL	163 ± 5

*P< 0.05 versus the other two groups

As shown by Table 3, differently from the reference

product, the nitroderivative of the invention was able to

inhibit blood hypertension induced by thrombosis induced by

L-NAME.

EXAMPLE 3B: STUDY OF OCULAR HYPOTENSIVE ACTIVITY AND OCULAR

SAFETY OF NO-ENA OR NO-TIM VERSUS ENALAPRIL OR TIMOLOL IN

RABBITS

In rabbits, the topical application of 100 μg of NO-ENA

or NO-TIM gave a more pronounced and more lasting (more than

6 hours) reduction of intraocular pressure (6-7 mmHg respe-

ctively) than the reference products timolol and enalapril.

Furthermore, for NO-TIM, the ratio between product concen-

trations in plasma (P) and aqueous humor (AH) versus timolol

was determined by an HPLC method. It was found that the P/AH

ratio for NO-TIM was 5.5 times lower than that for timolol,

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suggesting that the systemic absorption of the nitroderiva-
tive (and consequently any potential side effect from said
derivative) was markedly reduced compared to the reference
product.

EXAMPLE 3E: STUDY OF NO-ENA EFFECTS ON INDUCED BRONCHOCON-
STRICTION IN GUINEA PIGS VERSUS ENALAPRIL

Bronchoconstriction induced by capsaicin in Guinea pigs
is an animal model related to the ability of ACE (angioten-
sin-converting enzyme) inhibitors to cause cough in patients
(Subissi et al., J. Cardiovasc. Pharmacol.20/1, 139-146,
1992).

Adopted test conditions were as previously described by
Del Soldato et al.(J. Pharmacological Methods 5, 279, 1981).
Female Guinea pigs weighing 300 to 400 g were anaesthetised
by intraperitoneal injection of sodium 5,5-diethylbarbitu-
rate (200 mg/kg) and maintained under artificial respiration
at constant positive pressure. The right jugular vein was
incannulated for administering test compound. By a median
incision of the abdomen, the duodenum was removed and
through a small incision the tip of a suitable polyethylene
cannula was inserted and fixed. The other end of the can-
nula was connected to a syringe for intraduodenal admini-
stration of NO-ENA (10 mg/kg), enalapril (10 mg/kg) or vehi-
cle. 45 minutes later, 0.1 ml of capsaicin (1 μg/kg) was

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injected into the jugular vein of the animals. Before and
after injection of capsaicin, changes in the tidal area were
measured by a modified Konzett apparatus connected to a
suitable polygraphic amplifier (Hewlett Packard).

The results were calculated as the ratio of the respon-
ses obtained before and after administration of the test
compound, expressed as a % of the response obtained with the
vehicle alone, shown in Table 4.

TABLE 4

STUDY OF EFFECTS OF NO-ENA ON BRONCHOCONSTRICTION INDUCED IN
GUINEA PIGS VERSUS ENALAPRIL

TREATMENT	BRONCHOCONSTRICTIVE RESPONSE (%)
VEHICLE	100
NO-ENA	72
ENALAPRIL	327

As shown in Table 4, the nitroderivative of the inven-
tion reduced bronchoconstriction induced by capsaicin diffe-
rently from the reference product, which actually markedly
enhanced the bronchoconstrictive response.

CONCLUSIONS

As can be observed from the above examples, the nitro-
derivatives which are an object of the present invention
show marked antithrombotic and cardiovascular activity with
excellent safety when compared to reference products.

CLAIMS

1. Compounds, or their compositions, of the general formula:

$$A-(X_1-NO_2)_{t_o}$$
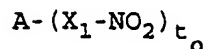
or their salts, where:

t_o is an integer equal to 1 or 2;

$A = RN_O$ where $N_O = (COX_u)_t-$ or $COON_1$ where t is an integer equal to zero or 1; u is an integer equal to 0 or 1;

$X = O$, NH, NR_{1c} where R_{1c} is a linear or branched alkyl having from 1 to 10 carbon atoms; N_1 is a linear or branched alkyl having from 1 to 10 carbon atoms or hydrogen;

R is chosen from the following groups:

* Group A)

Ia)
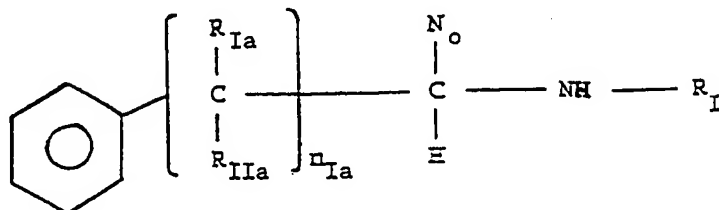
where R_{Ia} and R_{IIa} are equal or different one from the other and are H or a linear or whenever possible branched alkyl from 1 to 3 C atoms, preferably $R_{Ia} = R_{IIa} = H$; n_{Ia} is an integer from 1 to 6, preferably from 2 to

4;

R_I can be:

(X)

(XI)

(XII)

(XIII)

(XIV)

(XV)

(XVI)
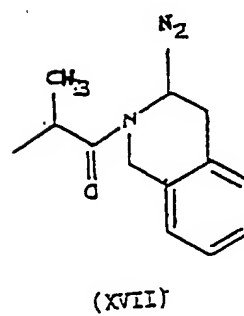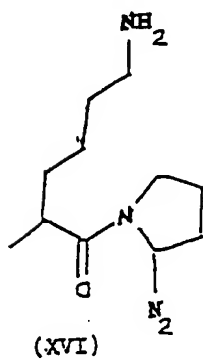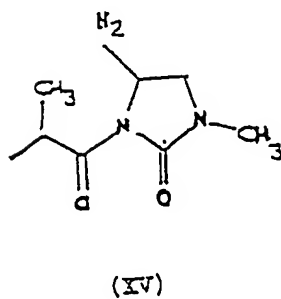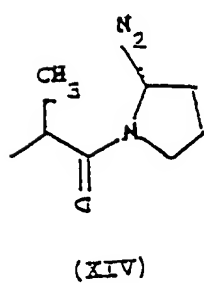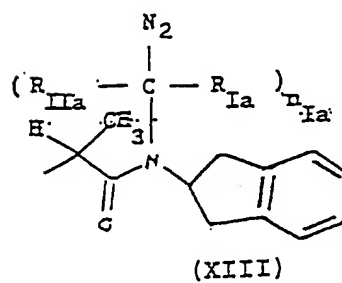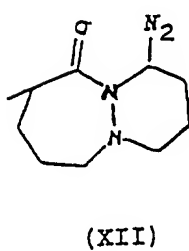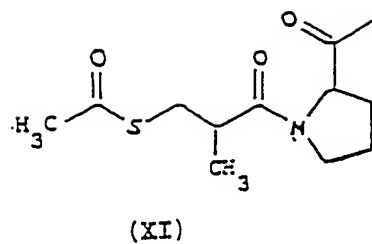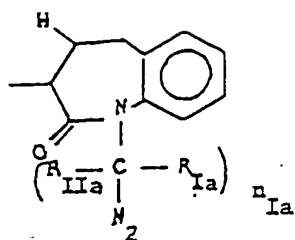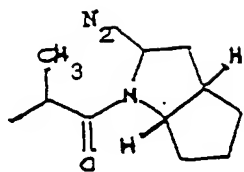
(XVII)

(XVIII)
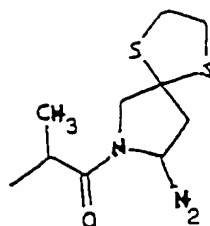
(XIX)

(XX)

(XXI)
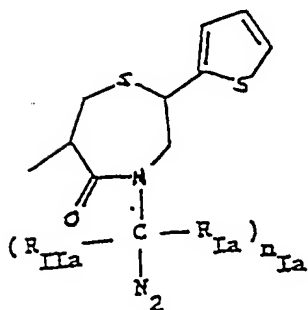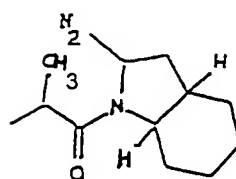
where N_2 has the same meaning as N_0; at least one of the groups N_0 or N_2 having one free valence capable of binding to X_1, (that is, $t = 1$),
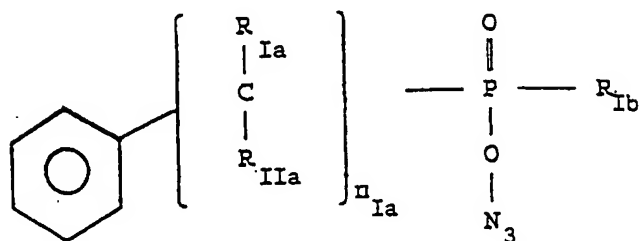
Ib)

R_{Ia}, R_{IIa}, n_{Ia} are as defined in Ia;

N_3 is H, $(CH_3)_2CH-CH-OCOCH_2CH_3$, or a free valence to

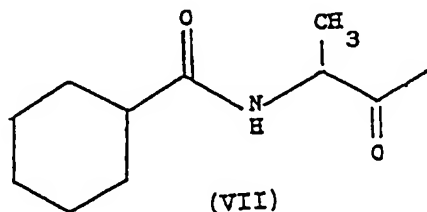which X_1 binds (that is, N_3 is absent);
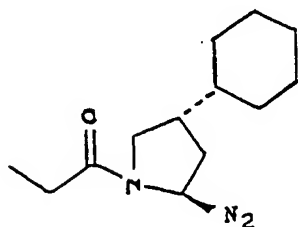
R_{Ib} is chosen from:

V)
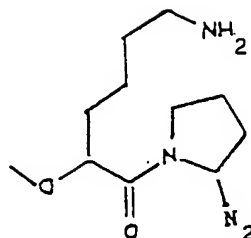
VI)

N_2 is as above defined, where at least one of the groups N_3 or N_2 has a free valence capable of binding to X_1 (when it is N_2, t = 1);

Ic) where t = 1
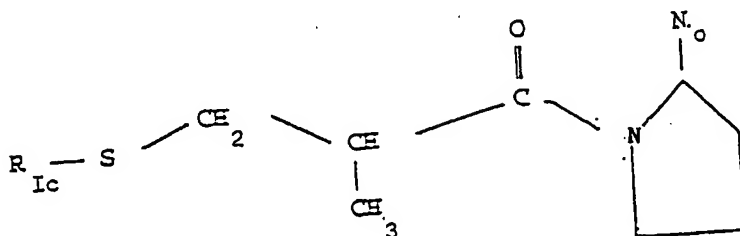
where N_0 is as above defined where t = 1, i.e., it has a free valence capable of binding to X_1;

R_{Ic} is chosen from H, $-COCH_3$, or

(VII)

Id)

where N_2 is as defined, and at least one of the groups N_2 has a free valence (t = 1) capable of binding to X_1;

* Group B

where t = 1 and u = 0;

IIa)
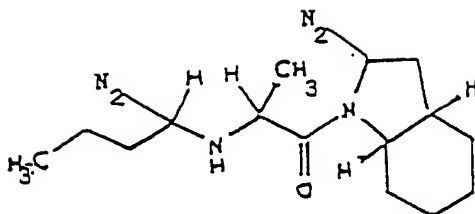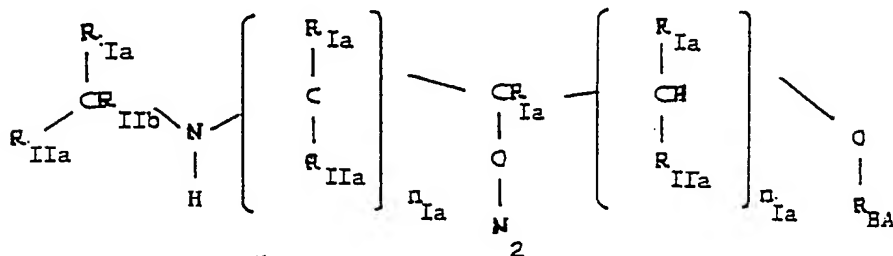
where R_{Ia}, R_{IIa} are as defined in Ia);

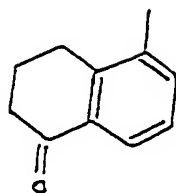R_{IIb} has the meaning of R_{Ia};
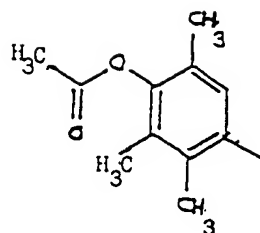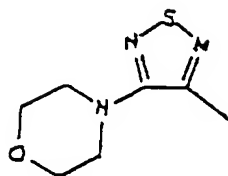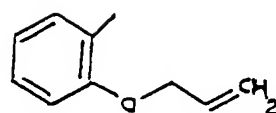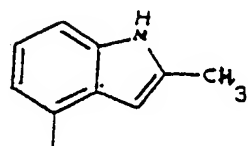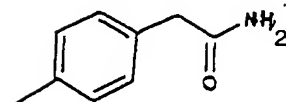
R_{BA} is chosen from:
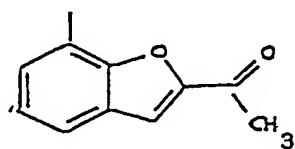
L)
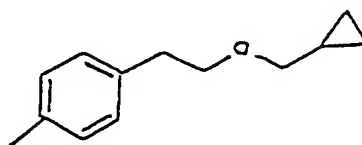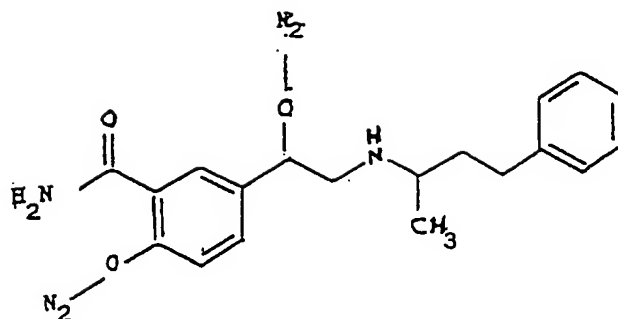

LI)
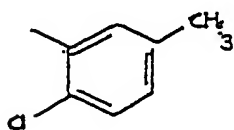
LII)
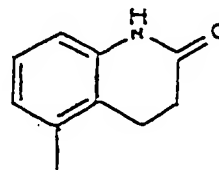
LIII)

LIV)
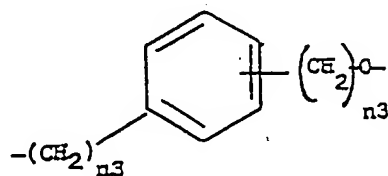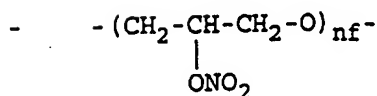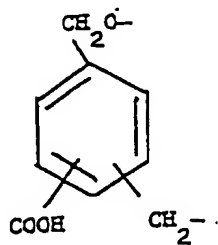
LV)

LVI)

LVII)

LVIII)

LIX)

IIb)

where in group B), N_2 is as above defined and at least
one of the N_2 groups has a free valence capable of bin-
ding to X_1, (that is, at least one N_2 substituent has t
= 1);

X_1 is a bivalent connecting bridge chosen from the fol-
lowing:

- YO where Y is a linear or whenever possible bran-
  ched C_1-C_{20} alkylene, preferably having from 2 to 5
  carbon atoms, or an optionally substituted cyclo-
  alkylene having from 5 to 7 carbon atoms;

- Y_1 chosen from

    -

  where n_3 is an integer from 0 to 3;

    —

- $-(CH_2-CH-CH_2-O)_{nf}-$
          |
        ONO_2

  where nf' is an integer from 1 to 6, preferably

  from 2 to 4.

- $-(CH-CH_2-O)_{nf}-$
  |
  R_{1f}
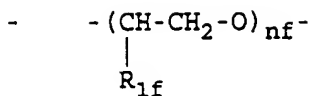
  where R_{1f} = H, CH_3 and nf is an integer from 1 to

  6, preferably from 2 to 4;

2. Compounds according to Claim 1, in which R, R_I, R_{Ib},

   R_{Ic}, R_{BA} and compounds Id) and IIb) are the residues of

   Alacepril, Benazepril, Captopril, Ceronapril, Cilaza-

   pril, Delapril, Enalapril, Enalaprilat, Fosinapril,

   Imidapril, Lisinopril, Quinapril, Ramipril, Spirapril,

   Temocapril, Trandolapril, Moveltilpril, Perindopril,

   Befunolol, Betaxolol, Bupranolol, Carteolol, Levobuno-

   lol, Metipranolol, Timolol, Oxprenolol, Mepindolol,

   Atenolol, Labetalol.

3. Compounds according to Claims 1 and 2, in which X_1 is
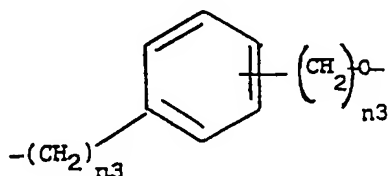
   chosen from

   - YO where Y is a linear or whenever possible bran-

     ched C_1-C_{20} alkylene, preferably having from 2 to 5

     carbon atoms, or an optionally substituted cyclo-

     alkylene having from 5 to 7 carbon atoms;

   - Y_1 chosen from

     -

where n_3 is an integer from 0 to 3;

4. Compounds or compositions in accordance with Claims 1 to 3 for use as medicaments.

5. Use of the compounds or compositions in accordance with Claims from 1 to 3 for the preparation of medicaments for application as antithrombotic agents.

6. Use of the compounds or compositions in accordance with Claims from 1 to 3 for the preparation of medicaments for application as antihypertensives.

7. Use of the compounds or compositions in accordance with Claims from 1 to 3 for the preparation of medicaments for application as cardioprotective agents.